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The Bumed News Letter is pleased to present an original contribution by Phillips, et al of the Navy Research Unit, Rockefeller Institute for Medical Research. A simple method for the determination of specific gravity of plasma and whole blood, it allows the determination of total proteins, hemoglobin and hematocrit by the single expedient of dropping these fluids into vials, each containing a different known concentration of copper sulfate. The specific gravity of the plasma and blood thus determined is then applied to line charts and total protein, hemoglobin and hematocrit values may be read off directly. The authors have so simplified the technic that it may be performed in almost any environment. Presentation of this original work represents a major contribution to essential laboratory procedures required by our newer knowledge of the pathological physiology and therapy of shock, burns, hemorrhage, the dysenteries, anemias, nephritides and other conditions which may alter either blood proteins or hemoglobin. It beautifully supplements recent developments in the plasma therapy of war injuries.

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The Copper Sulfate Method for Measuring Specific Gravities of Whole Blood and Plasma: This method makes it possible with three or four drops of blood and no apparatus except a medicine dropper and small bottles of copper sulfate solution to determine the specific gravity of the blood, and from it the hemoglobin content within 10 per cent. By examining in a like manner the serum or plasma from the same blood one can determine also the plasma protein concentration and increase the accuracy of the hemoglobin estimation to ± 2 per cent. To measure the blood and plasma gravities and calculate the plasma proteins, hemoglobin and hematocrit on a line chart requires about 2 minutes.

The results can be used as follows:

1. To assist in ascertaining the results of hemorrhage.

2. To estimate decreases in plasma volume from hemoglobin increases, and to decide whether the plasma volume decrease is due to loss of water (dehydration of cholera, dysentery, exposure), or to loss also of plasma proteins (extravasation in burns, trauma, etc.).

3. To assist in deciding whether blood replacement therapy requires administration of saline solution or plasma or whole blood.

4. To follow the results of such therapy and decide whether it has been adequate, and when it must be repeated.

5. If the number of cases exceeds the amount of blood and plasma available, the method will assist in deciding which cases must receive it and which may be able to do without.

6. Beside these acute conditions, the method will assist in diagnosing the different types of anemia, and in detecting various pathological conditions, partially summarized in a later section, in which the plasma proteins become diluted or concentrated.

The utility of blood and plasma concentration measurements is well recognized in the diagnosis and treatment of shock from wounds, burns, hemorrhage, etc., and in other conditions in which plasma proteins or hemoglobin are affected. However, the gravity methods hitherto used which are of sufficent accuracy, such as the gravimetric, the falling drop method and the gradient tube require precision instruments on stable bases. None of these methods, for example, could well be used on shipboard.

Principle of the Method: The technic consists of letting drops of plasma or whole blood fall into a graded series of solutions of copper sulfate of known specific gravity, and noting whether the drops rise or fall in the solutions. Each drop on entering the solution becomes encased in a sack of copper-proteinate, and remains as a discrete drop without change of gravity for 15 or 20 seconds, during which its rise or fall reveals its gravity relative to that of the solution. The size of the drops does not have to be constant, hence no special pipette is needed for delivering the drops. No temperature correction is needed, because the temperature coefficient of expansion of the copper sulfate solutions approximates that of blood and plasma. This method is capable of measuring gravities to $\pm$ 0.00005, which is ten times the accuracy required. The copper sulfate solution automatically cleans itself after each test, because within a minute or two after the test is completed the material of the drop settles to the bottom as a precipitate.

The principle of dropping blood into a series of solutions of known gravity has been applied for decades but has never achieved entire success. Mixtures of organic liquids have been used, e.g., benzene and chloroform, but they were liable to change in specific gravity due to differential evaporation of the components. They also have temperature coefficients of expansion several times that of water or blood and cannot be used without accurate temperature regulation. In addition, some of them give rise to toxic

and to explosive vapours. Dispersion of blood occurs too rapidly in the usual aqueous salt or glycerol solutions to enable small gravity differences to be measured easily and accurately. The use of aqueous standard solutions which have protein-coagulating power overcomes these difficulties.

For accurate work, viz., gravities within ± 0.0002, a series of copper sulfate solutions graded at intervals of 0.001 in specific gravity is used; twenty solutions cover the plasma range 1.015 - 1.035 and forty cover the whole blood range, 1.035 - 1.075. For rougher work with gravities accurate to ± 0.001, sixteen solutions at intervals of 0.004 suffice to cover the entire range of blood and plasma.

Procedure for Gravity Analysis of Blood and Plasma: The drop of serum, plasma or whole blood is delivered from a height of about 1 cm. above the solution from a pipette, or from a needle attached to a glass syringe. It is preferable to use small drops for the reason that they permit more tests before the standard solution must be changed. Therefore a pipette with a fine tip is preferable to a coarse one for delivering the drop. Greasing the sides of the tip with vaseline also reduces the size of the drop, especially if the vaseline is mixed with a little caprylic alcohol.

The delivered drop breaks through the surface film of the solution and penetrates 2 to 3 cms. below the surface; within 5 seconds the momentum of the fall is lost and the drop then either begins to rise, or becomes stationary, or continues to fall. The gravity of the drop relative to the solution does not change appreciably until the drop has been immersed in the solution for about 15 seconds, and there is ample time to note its behavior during this interval. If the drop is lighter than the test solution it will rise, perhaps only a few millimeters during this interval, and may begin to sink immediately afterwards. If the drop is of the same gravity as the standard test solution it will become stationary for this interval and then fall. If the drop is heavier it will continue to fall during the interval. In summary, the behavior during the 10 seconds after the drop has lost the momentum of its fall into the solution indicates whether the drop is lighter or heavier than the test solution; if it rises at all during this period it is lighter than the standard.

Example: The following example shows how, by bracketing on the probable extremes of a plasma's gravity range and then testing intermediate points, one can find the correct gravity with not more than 4 drops to within ± 0.0002.

The plasma was expected to be of normal or greater concentration. Four successive drops gave the following results, in which the figures indicate the gravities of the standards, and + or - indicates that the plasma was heavier or lighter than the standard: 1.027, +; 1.031, -; 1.029, +; 1.030, - . The plasma was heavier than 1.029 and lighter than 1.030, and could therefore be placed at 1.0295 with an error less than ± 0.0005.

By noting the relative rate of rise or fall in the two adjacent solutions, 1.029 and 1.030, it was further obvious that the plasma was nearer 1.029

than 1.030. Being less than 1.0295 and greater than 1.0290 it could be placed at 1.0292 or 1.0293 with an error not greater than ± 0.0002.

Approximate Field Determinations: For field work it may suffice to determine the gravities to ± 0.001. For this only 16 standard solutions with gravity intervals of 0.004 covering the range from 1.016 to 1.076 will be needed. An error of 0.001 in plasma gravity affects plasma proteins by 0.3 gram per 100 cc.; additive errors of 0.001 in the gravities of both plasma and whole blood affect hemoglobin results by 5 per cent.

Example: The plasma tested was lighter than 1.028 and heavier than 1.024. By observing the behavior of the drop in the two solutions it was noted to be closer to 1.028 than to 1.024 and hence could be placed at 1.027 with an error not greater than ± 0.001.

Calculations: Line charts for the conversion of plasma and whole blood gravities to plasma protein concentration, hemoglobin concentration and hematocrit percentages have been prepared by standard methods, and are given in Figs. 1 and 2.

The calculations are made by laying a straight edge or stretched thread as directed on the charts.

If anticoagulant other than heparin is used the correction indicated at the bottom of the line chart, Fig. 2 should be applied.

For men the normal values are taken from precise measurements made for this purpose on the blood and plasma of 20 normal men. For women normal hemoglobin and cell volume are taken as 90 per cent of the normal for men.

Precautions:

1. A tourniquet applied for more than 1 minute will cause measurable hemoconcentration.

2. Anticoagulants.

(a) Heparin, 0.2 mgm. per cc. blood, does not change gravities.
(b) Oxalate mixture, 1 mgm. per cc. blood, increases gravities of both plasma and whole blood about 0.0004. This effect is negligible for most purposes; it raises calculated plasma protein and hemoglobin values each by only 0.1 gm. per 100 cc. When, however, less than 4 cc. of blood is added to a tube containing 5 mgm. of oxalate mixture, corrections are made by subtracting from both the whole blood and the plasma gravities 0.0007 if the blood volume is 3 cc., 0.0010 if it is 2 cc.. 0.0020 if it is 1 cc.
(c) Citrate introduces large errors.

3. Analysis without Anticoagulants. Anticoagulants can be dispensed with if the gravity of the whole blood is determined by dropping it directly from the syringe needle into the standard copper sulfate solutions. The

remainder of the blood can be transferred to a tube and permitted to clot. From the centrifuged or sedimented material a few drops of serum are drawn up into a dropper and are used for serum gravity determination.

4. Renewing standards. Renewal is needed when about 1 small drop of blood or plasma per cc. of standard solution has been added. This will decrease the gravity of the solution by 0.0005. A 4-ounce bottle of standard serves for about 100 tests. One extra standard of gravity 1.027 is prepared and to the solution one-fortieth of its volume of plasma is added dropwise. When the volume of precipitate in the bottom of a repeatedly used standard equals that in this control bottle the standard is renewed.

5. Surface film. Occasionally a drop will fail to make a clean break through the surface film of the copper sulfate solution, and remain attached by a tentacle to the film. In this case the drop is detached from the film by tapping the tube, and a fresh drop is tried.

After each test one makes sure that the surface film is left clean and free from fragments. If any are left on the film they are likely to prevent a clean break-through of the drop in the next test. Fragments caught in the surface film can usually be detached by tapping the tube; they then sink to the bottom. Sometimes, however, a fragment of fatty nature or holding a bubble will continue to float on the surface. Such fragments are removed with a wooden applicator stick.

6. Temperature. The method requires but little attention to temperature.

(a) Change of temperature of standard solutions of ± 10°C. changes gravities not more than ± 0.0002, because the temperature effect is nearly the same on the gravities of standards as of blood.

(b) Blood drawn from a vein into a syringe can be delivered directly from the syringe needle into standard solutions at 20° or above without error exceeding 0.0003 in the gravity measurement. Blood or plasma drawn into a medicine dropper and delivered into the copper sulfate solutions should be within 5° Centigrade of the temperature of the solutions.

(c) Convection currents in the standard solutions could introduce false readings. Do not bring cold bottles into a warm room and use at once. Do not leave bottles near stove, on window sill, etc. Hold bottles only by top when using, not by sides.

Bottles or Tubes for the Standard Copper Sulfate Solutions: For precise determination (gravities to ± 0.0002) 70 "oval prescription" bottles of 4, 2, or 1-ounce capacity. For field determination (gravities to ± 0.001) 16 bottles suffice.

Rubber stoppers, corks, or screw-tops are preferable to glass stoppers. The bottles are labeled with gravity figures from 1.008 to 1.075, with the labels where they can be read from above, and are arranged in ranks of five, for convenience in selecting desired solutions. Seventy 4-ounce bottles thus arranged occupy a space about 10 x 20 inches.

For laboratory use the 4-ounce bottles are preferable because they permit about 100 analyses, without replacing the solution.

For portable sets the 1 or 2 ounce bottles may be used. It is convenient to bind these in sets of 5 with transparent "Scotch tape."

Test tubes of 16 x 150 mm. size, or preferably 25 x 200, heavy-walled, can be used in place of bottles.

Apparatus for Preparing the Copper Sulfate Solutions: One volumetric flask of 100 cc. capacity and one burette, preferably also of 100 cc. capacity, for preparing standard copper sulfate solutions to be stored in 4-ounce bottles. If 2-ounce bottles are used the flask and burette should be of 50 cc. capacity; if 1-ounce bottles are used the flask and burette should be of 25 cc. capacity.

Three 4-liter bottles.

One 1-liter volumetric flask.

One 500 cc. graduated cylinder.

One 7-inch funnel, and cotton or filter paper.

One thermometer, 0°C. - 40°C. or corresponding Fahrenheit.

Reagents: Oxalate mixture. 3 gm. ammonium oxalate and 2 gm. potassium oxalate are dissolved in 250 cc. H_2O. 0.25 cc. are pipetted into round-bottom, heavy-walled, 15 x 125 mm., pyrex tubes. The tubes are placed on their sides, to spread solution in a film, in an incubator (not over 50°C.) and dried. Mark the outside of the tubes at the 5 cc. level with a glass-marking pencil.

The oxalate mixture (Heller and Paul, J.Lab.Clin.Med., 19, 777 (1934)), disturbs cell and plasma gravities less than either potassium or ammonium oxalate alone.

Crystalline copper sulfate, $CuSO_4.5H_2O$. This is preferably purchased in the form of "fine crystals." Otherwise it must be pulverized before using. Four pounds provide a complete set of 100 cc. standard solutions. Ten pounds will probably suffice a laboratory for a year.

Preparation of Copper Sulfate Solutions: Saturated copper sulfate solution. This solution is used to prepare a stock solution of gravity 1.100 ± 0.0003. Use of a solution saturated at a known temperature affords a precise means of preparing the stock solution without a balance. The saturated solution is prepared as follows:

Four pounds of "fine crystals," or pulverized copper sulfate are placed in a 4-liter bottle. About 2500 cc. of distilled water is added, and the bottle is stoppered and shaken vigorously for a total of 5 minutes, which need not be continuous. (Three minutes has been found sufficient, even at 0°C.,

to saturate this solution if the sulfate is well pulverized.) As soon as the shaking is finished the temperature of the solution is taken to the nearest half degree Centigrade and is recorded. (It will be a little cooler than the water was before the saturation, because the saturation process absorbs heat.) The solution is immediately decanted off the crystals and is filtered, to remove fine suspended crystals, through cotton or dry filter paper into a clean, dry 4-liter bottle. The solution is at once used to make up a stock solution of gravity 1.100. (It is preferable not to let the saturated solution stand long before using, as if it cools some of the copper sulfate may crystallize and change the concentration.) The undissolved sulfate can be used again.

Two and a half liters of the saturated solution suffice for more than 4 liters of the stock solution of gravity 1.100, and this in turn is sufficient for a complete set of 70 standard solutions of 100 cc. volume each, with enough surplus to provide replacements for the standards which are most used. Smaller or larger amounts of the saturated solution can be made by using proportional amounts of copper sulfate and water.

Stock Copper Sulfate Solution of Gravity 1.100. (Gravity figures in this report were determined as the ratio of the weight of copper sulfate solution to the weight of an equal volume of water at the same temperature.) The volume of saturated solution indicated in Table 1 is measured in a 500 cc. graduated cylinder and poured into a 1-liter volumetric flask. The upturned cylinder is allowed to drain into the flask for 30 seconds. The flask is then filled to the mark with water, and is inverted several times to mix the solution. The mixing results in a contraction, so that the meniscus now falls below the mark. The flask is let stand for a minute until the solution drains down from the neck. Then enough additional water is added to bring the volume to 1 liter, the solution is mixed, and then poured into a clean, dry, 4-liter bottle. The same 1-liter volumetric flask is used to prepare 3 more liters of the stock copper sulfate solution of gravity 1.100. Each time before the flask is used again it is rinsed with water and the rinsings are discarded.

It is desirable that the saturated solution, the stock solution and the standard solutions next described shall all be prepared at within 5°Centigrade of the same temperature. The coefficients of expansion of the saturated and stock copper sulfate solutions are slightly but definitely greater than that of water, so that if the saturated solution and stock solution were prepared at 35°C. and the standard solutions at 20°C. the standards would have more copper sulfate than intended, enough to increase the gravity by about 0.001.

Preparation of Standard Solutions in 100 cc. Portions. The standard solutions are prepared in 100 cc. portions when 4-ounce bottles are available for storage.

For the standard of 1.075 gravity, 74 cc. of stock solution of gravity 1.100 are measured from the burette into the 100 cc. flask, the flask is filled to the mark with water, and the solution is mixed and transferred to a labeled 4-ounce bottle, which is stoppered to prevent evaporation.

To prepare the standard of gravity 1.074, the 100 cc. flask is rinsed once with water and the burette is refilled from a 250 cc. Erlenmeyer flask containing the stock solution. Then 73 cc. of the stock solution are measured into the volumetric flask and diluted to 100.

The same procedure is carried through for preparation of the entire series down to 1.015, which covers the extreme ranges for blood and plasma. If gravities on ascitic fluid and transudates may be desired also, the series is extended to 1.008. For each standard the number of cc. of stock solution less by 1 than the number indicated in the second and third decimal places of the desired gravity is measured into the rinsed 100 cc. flask and diluted to the mark.

If there were no contraction when the stock solution is mixed with water one would dilute 75 cc. of the stock to 100 cc. to get a gravity of 1.075 etc. However, there is a contraction which is empirically corrected by taking 1 cc. less of the stock. It happens conveniently that the same 1 cc. correction serves for the entire range, 1.075 to 1.008, over which its use yields gravities correct within ± 0.0003.

For field work with gravities accurate to ± 0.001, sixteen standard solutions varying by 0.004 and covering the range of 1.016 - 1.076 are prepared.

Preparation of Standard Solutions in 50 or 25 cc. Portions. The volumes of stock solution indicated in Table 2 are measured from 50 or 25 cc. burettes into 50 or 25 cc. flasks.

Equations on Which the Line Charts are Based:

G_P = Specific gravity of plasma; G_B = Specific gravity of whole blood.

1.0970 = average specific gravity of normal cells.

(1) Plasma protein (gm. per 100 cc. plasma) = $343\ G_P - 1.0070$

(2) Hematocrit (cc. cells in 100 cc. whole blood) = $100\ \frac{G_B - G_P}{1.0970 - G_P}$

(3) Oxygen capacity (cc. O_2 bound in 100 cc. whole blood) = $46.1\ \frac{G_B - G_P}{1.0970 - G_P}$

(4) Hemoglobin (grams per 100 cc. whole blood) = $33.9\ \frac{G_B - G_P}{1.0970 - G_P}$

46.1 represents mean cc. of oxygen bound by hemoglobin in 100 cc. of cells. 33.9 indicates the mean grams of hemoglobin in 100 cc. of cells.

The constants 1.0970, 46.1 and 33.9 were found by precise oxygen capacity, hematocrit, and gravity determinations on the blood of 20 normal men.

The above equations can be used to calculate results in case the line charts are not available.

Effects of Cell Abnormalities on Hematocrits and Hemoglobins Calculated from Gravities: In blood with cells of abnormal hemoglobin content, as in hypochromic anemias, the calculation of hematocrits and hemoglobins from gravity values is not as accurate as in blood with cells of normal composition. However, we have seldom encountered a blood in which the error in hemoglobin was greater than 1 volume per cent O_2 capacity, or 0.7 gm. of hemoglobin per 100 cc.

The reason why even marked abnormalities in the hemoglobin content of the cells do not cause greater errors in blood hemoglobin concentrations calculated from gravities is presumably the fact that changes in cell gravity and cell hemoglobin content are parallel. The calculations (Equations 2, 3, and 4) assume that the cell gravity remains constant at 1.0970 and the hemoglobin content at 33.9 grams per 100 cc. of cells. When both these values change in the same direction their changes in Equation 4 partly cancel each other in their effects on hemoglobin calculated from gravity values. The hematocrit values calculated from gravities in hypochromic anemia may be less accurate.

Pathological Conditions Affecting the Plasma Protein Concentration: The different types of anemia and polycythemia are so well known in their effects on blood hemoglobin concentration that it is unnecessary to discuss them here. However, the numerous conditions that cause a decrease or increase in the plasma protein concentration are not so well known. The most adequate summary of them is the recent one by Kagan (Southern Med. J., Birmingham, Alabama, 36, 234 (1943)) and Table 3 is drawn chiefly from his data.

So far as the clinical utility of plasma protein determinations is concerned, one may say that in acute conditions the determinations may serve chiefly as guides in therapy to correct abnormal volume or protein concentration of the plasma, while in chronic conditions the determinations serve more as aids in diagnosis of pathological conditions.

In diagnosis, an abnormal plasma protein concentration is definite proof that one of the factors controlling the concentration has been disturbed.

However, a normal protein concentration is not final proof that the factors controlling the concentration are all working normally, for there may be abnormalities with opposite effects which balance; in such a case the plasma gravity fails to indicate the pathological conditions that nevertheless exist. Such a balance may occur in either an acute condition, such as hemorrhage, or a chronic one, such as liver cirrhosis or diabetes.

Hemorrhage at first causes a general hemodilution, affecting both plasma proteins and hemoglobin as interstitial fluid enters the circulation from the tissue spaces to replace the lost blood. If the hemorrhage leads to shock, however, hemoconcentration may set in, with decrease of blood volume and return of the plasma and hemoglobin concentrations towards or even above normal.

Liver cirrhosis retards albumin synthesis, but may increase globulin formation, with a resultant normal total protein concentration in the plasma.

Chronic diabetes tends to produce malnutrition and deficit of plasma albumin and total protein, but in acidosis, dehydration may raise the total protein concentration to normal or above.

It is evident from the above that, although blood and plasma concentration data provide information concerning a patient's condition that can not be obtained by clinical inspection alone, nevertheless the concentration data can not be applied by any rule of thumb, but must be considered together with the history and other available data in deciding on the patient's condition and the indicated therapy.

Burns, Post-Operative Conditions, and Traumatic Shock: In these three conditions, with their rapid and often critical shifts of hemoconcentration, estimations of the plasma proteins and the hemoglobin by the gravity technic are of especial utility, both in detecting the changes and in guiding the therapy. For data on which the following summary is chiefly based the authors are indebted to Dr. John Scudder (personal communication).

Burns. Seepage of plasma from the denuded areas causes a decrease both in the volume and the protein concentration of the plasma. In consequence the plasma proteins fall while the hemoglobin rises.

In some cases during the first hours a loss of water from the blood occurs to such an extent that the plasma proteins show a transitory rise. This, however, is followed by the fall, described above, as the effects of protein loss by seepage accumulate. The occasional initial dehydration of the blood seems to be due partly to passage of water from blood to tissues, as it may occur when there is no marked external loss of fluid, as by vomiting and sweating. The hemoglobin rises during this stage, as well as during the subsequent stage when the effects of seepage dominate.

To guide plasma replacement therapy it is desirable to determine plasma and blood gravities one or more times daily for several days.

Post-operative conditions. In the post-operative period dehydration of the blood is likely to increase both the plasma protein and the hemoglobin concentrations. The dehydration may go so far as to produce uremia. Repeated saline injections may be required to replace fluid. However, if too much saline is administered by unregulated infusions, hemodilution and a waterlogged, edematous state of the tissues may result which is as undesirable as the dehydration.

If changes in blood gravity are followed infusions can be so regulated that error in either direction is avoided.

For repeated injections it is safer to use Hartmann's (J. Am. Med. Asso., 103, 1349 (1934)) solution rather than 0.9 per cent NaCl. Hartmann's solution contains 6 gm. NaCl, 4 gm. sodium lactate, 0.4 gm. KCl, 0.2 gm. $CaCl_2$. $2H_2O$, and 0.2 gm. $MgCl_2$. $6H_2O$, per liter. Repeated infusion of NaCl alone may result in fall of plasma bicarbonate, potassium, and calcium. The loss of bicarbonate is particularly undesirable, because it exacerbates the acidosis that may be produced by either shock or anesthesia. The lactate in Hartmann's solution is equivalent to 75 per cent as much bicarbonate, because it is burned to bicarbonate in the body.

Besides their use in directing fluid replacement therapy, blood gravity measurements can assist in detecting certain post-operative complications. Peritonitis, intestinal fistulae, abscesses, and pancreatitis all cause the same blood changes seen after burns, viz., fall in plasma proteins and rise in hemoglobin. (In pancreatitis an initial period of increased plasma proteins may intervene, as in some cases of burns.) In perforations of the gastro-intestinal tract the plasma proteins first rise, then fall. The behavior of the hemoglobin depends on the extent of hemorrhage, the variable effects of which have already been discussed. A fall in hemoglobin indicates marked loss of blood, but maintenance of hemoglobin concentration is not certain evidence against such loss.

Traumatic shock. Varying degrees of dehydration, of plasma protein seepage from injured vessels, tissues, and surfaces, and of internal and external hemorrhage, can combine to produce such unpredictable effects on blood volume and concentration that observations on the blood are especially needed, together with careful interpretation. Marked changes in hemoglobin or plasma protein concentration provide clear guidance. But it is possible for blood dehydration so to balance losses of plasma and hemoglobin that their concentrations remain, or return to, normal. Such a paradoxical condition is to be suspected when the clinical and vascular signs of shock accompany normal gravity values. The circulating volume of blood is then low, despite the normal concentrations, and transfusion of whole blood in preference to plasma is indicated.

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(From the United States Navy Research Unit at the Hospital of the Rockefeller Institute for Medical Research, Commanding Officer, Commander Thomas M. Rivers.)

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Table 1

(Copper Sulf. Method for Sp. Gr. of Blood and Plasma, Phillips, et al.)

Cc. of Saturated Copper Sulfate Solution to be Diluted to 1-liter to Give the Stock Solution of Specific Gravity 1.100

Temperature in °C. or °F. refers to the temperature of the saturated solution at the time of saturation (end of shaking for 5 minutes).

Cc. = cc. of the saturated copper sulfate solution to be diluted to 1000 cc. to give the stock solution of specific gravity 1.100.

Temperature °C.	Temperature °F.	cc.	Temperature °C.	Temperature °F.	cc.	Temperature °C.	Temperature °F.	cc.
10.0	50.0	578	20.0	68.0	488	30.0	86.0	425
10.5	50.9	573	20.5	68.9	484	30.5	86.9	423
11.0	51.8	568	21.0	69.8	480	31.0	87.8	420
11.5	52.7	563	21.5	70.7	477	31.5	88.7	417
12.0	53.6	558	22.0	71.6	473	32.0	89.6	414
12.5	54.5	553	22.5	72.5	469	32.5	90.5	412
13.0	55.4	548	23.0	73.4	466	33.0	91.4	409
13.5	56.3	543	23.5	74.3	463	33.5	92.3	406
14.0	57.2	539	24.0	75.2	460	34.0	93.2	403
14.5	58.1	534	24.5	76.1	456	34.5	94.1	401
15.0	59.0	529	25.0	77.0	453	35.0	95.0	398
15.5	59.9	525	25.5	77.9	450	35.5	95.9	395
16.0	60.8	521	26.0	78.8	447	36.0	96.8	392
16.5	61.7	516	26.5	79.7	445	36.5	97.7	390
17.0	62.6	512	27.0	80.6	442	37.0	98.6	387
17.5	63.5	508	27.5	81.5	439	37.5	99.5	384
18.0	64.4	504	28.0	82.4	436	38.0	100.4	381
18.5	65.3	500	28.5	83.3	434	38.5	101.3	379
19.0	66.2	496	29.0	84.2	431	39.0	102.2	376
19.5	67.1	492	29.5	85.1	428	39.5	103.1	373
20.0	68.0	488	30.0	86.0	425	40.0	104.0	370

Table 2

(Copper Sulfate Method for Sp. Gr. of Blood and Plasma, Phillips, et al.)

Cc. of Stock Copper Sulfate Solution to be Diluted to 100 cc., 50 cc. or 25 cc. when Standard Solutions Accurate to ± 0.0001 are desired.

G	100	50	25	G	100	50	25
1.008	7.33	3.67	1.84	1.042	41.00	20.50	10.25
9	8.32	4.16	2.08	43	42.00	21.00	10.50
10	9.31	4.66	2.33	44	43.00	21.50	10.75
11	10.30	5.15	2.58	45	44.00	22.00	11.00
12	11.29	5.65	2.83	46	45.00	22.50	11.25
13	12.28	6.14	3.07	47	46.00	23.00	11.50
14	13.27	6.64	3.32	48	47.00	23.50	11.75
15	14.26	7.13	3.57	49	48.00	24.00	12.00
16	15.25	7.63	3.82	50	49.00	24.50	12.25
17	16.24	8.12	4.06	51	50.00	25.00	12.50
18	17.23	8.62	4.31	52	51.00	25.50	12.75
19	18.22	9.11	4.56	53	52.00	26.00	13.00
20	19.21	9.61	4.81	54	53.00	26.50	13.25
21	20.20	10.10	5.05	55	54.00	27.00	13.50
22	21.19	10.60	5.30	56	55.00	27.50	13.75
23	22.17	11.09	5.55	57	56.00	28.00	14.00
24	23.15	11.58	5.79	58	57.00	28.50	14.25
25	24.14	12.07	6.04	59	58.00	29.00	14.50
26	25.12	12.56	6.28	60	59.00	29.50	14.75
27	26.10	13.05	6.53	61	60.00	30.00	15.00
28	27.08	13.54	6.77	62	61.00	30.50	15.25
29	28.06	14.03	7.02	63	62.00	31.00	15.50
30	29.04	14.52	7.26	64	63.00	31.50	15.75
31	30.02	15.01	7.51	65	64.00	32.00	16.00
32	31.00	15.50	7.75	66	65.00	32.50	16.25
33	32.00	16.00	8.00	67	66.00	33.00	16.50
34	33.00	16.50	8.25	68	67.04	33.52	16.76
35	34.00	17.00	8.50	69	68.08	34.04	17.02
36	35.00	17.50	8.75	70	69.12	34.56	17.28
37	36.00	18.00	9.00	71	70.16	35.08	17.54
38	37.00	18.50	9.25	72	71.20	35.60	17.80
39	38.00	19.00	9.50	73	72.24	36.12	18.06
40	39.00	19.50	9.75	74	73.27	36.64	18.32
41	40.00	20.00	10.00	75	74.30	37.15	18.58

G = specific gravity of standard solution.
100 = Cc. of 1.100 stock solution diluted to 100 cc.
50 = Cc. of 1.100 stock solution diluted to 50 cc.
25 = Cc. of 1.100 stock solution diluted to 25 cc.

Table 3

(Copper Sulfate Method for Sp. Gr. of Blood and Plasma, Phillips, et al.)

Conditions Causing Abnormally High or Low Concentrations of Protein in the Plasma.

General Causes

High Concentrations.	Low Concentrations.
Due usually either to dehydration or to increased globulins. Increased globulins are common in chronic infections and diseases of the reticulo-endothelial system.	Due usually either to mechanical loss of proteins by extravasation or renal excretion, or to decreased albumin formation as the result of malnutrition or liver disease.

Specific Causes

High Concentrations.

1. Dehydration.

a. Insufficient fluid intake, especially when accompanied by exposure, as in open boats.
b. Fluid loss: Intestinal obstruction and fistulae; diarrhea, especially in infants, also cholera and dysentery; vomiting; severe diabetic acidosis; intense heat and exertion; Addison's disease; shock, surgical and traumatic; burns, first few hours (some cases); fulminant infections.

2. Diseases involving the reticulo-endothelial system. (High globulin.)

Multiple myeloma.
Monocytic leukemia.
Liver cirrhosis and cancer.

3. Chronic infections. (High globulins.)

Ulcerative tuberculosis; syphilis; lymphopathia venerum; subacute bacterial endocarditis; periarteritis nodosa; lupus erythematosis; rheumatoid arthritis; Boeck's sarcoid; leprosy; kala azar; schistosomiasis; filariasis; trypanosomiasis.

Low Concentrations.

1. Physical escape of plasma proteins from the circulation.

Hemorrhage, acute or chronic. Weeping wounds or skin lesions (burns). Albuminuria. Shock, surgical and traumatic.

2. Malnutrition. (Low albumin.)

Low protein diet.
Vitamin deficiencies, beri-beri, pellagra, etc.
Incomplete absorption, sprue.
Cancer of stomach, pancreas.
Pernicious anemia.
Diabetes mellitus, unregulated.
Hyperthyroidism.
Toxemias of pregnancy.

3. Conditions in which albumin synthesis is retarded, presumably because of liver damage. (Low albumin.)

Cirrhosis and cancer of liver.
Chronic poisoning, benzene, phosphorus, etc.

Fig. 1

(Copper Sulfate Method for Sp. Gr. of Blood and Plasma, Phillips, et al.)

Line chart for calculating plasma proteins, hemoglobin and hematocrit from gravities of plasma and blood

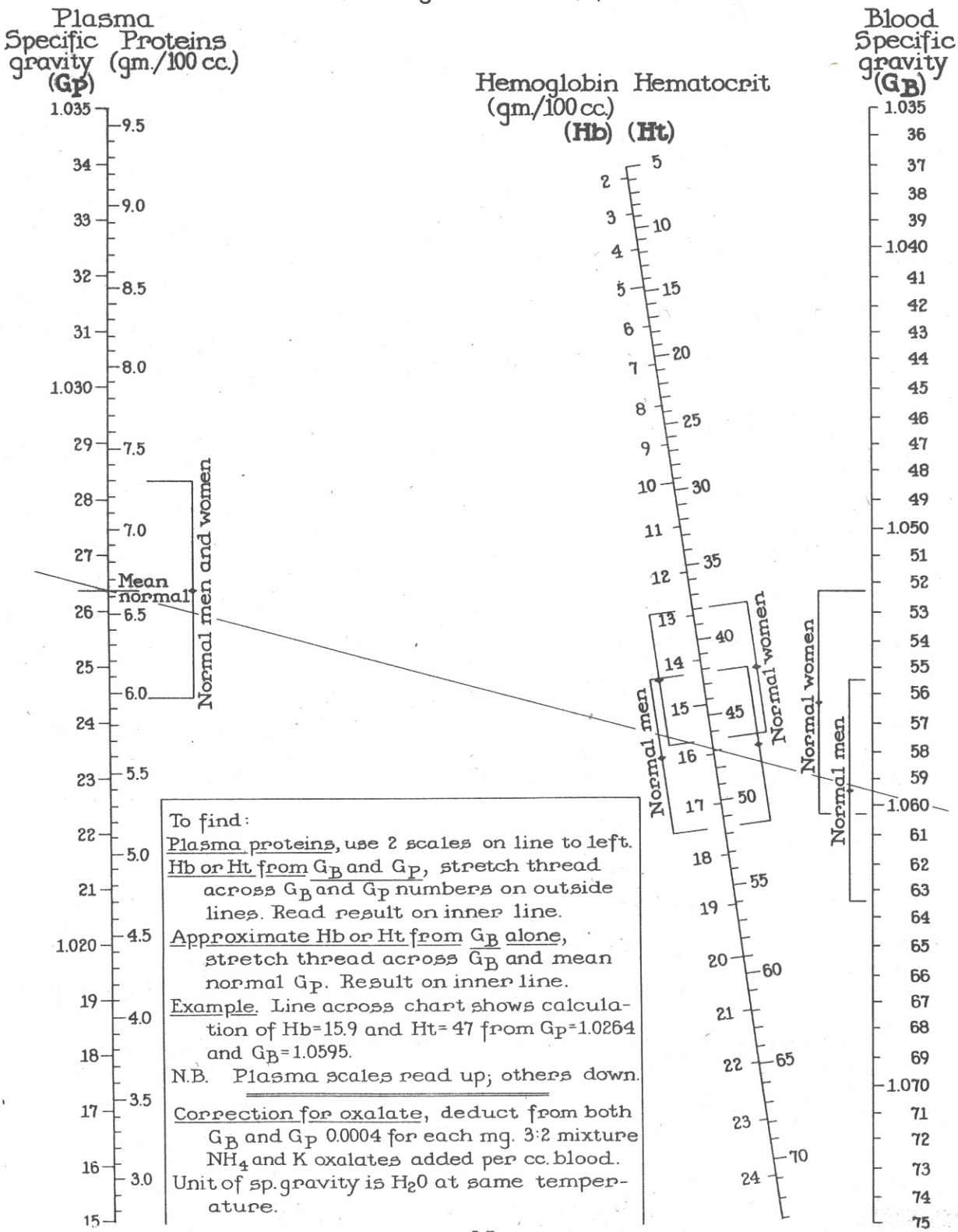
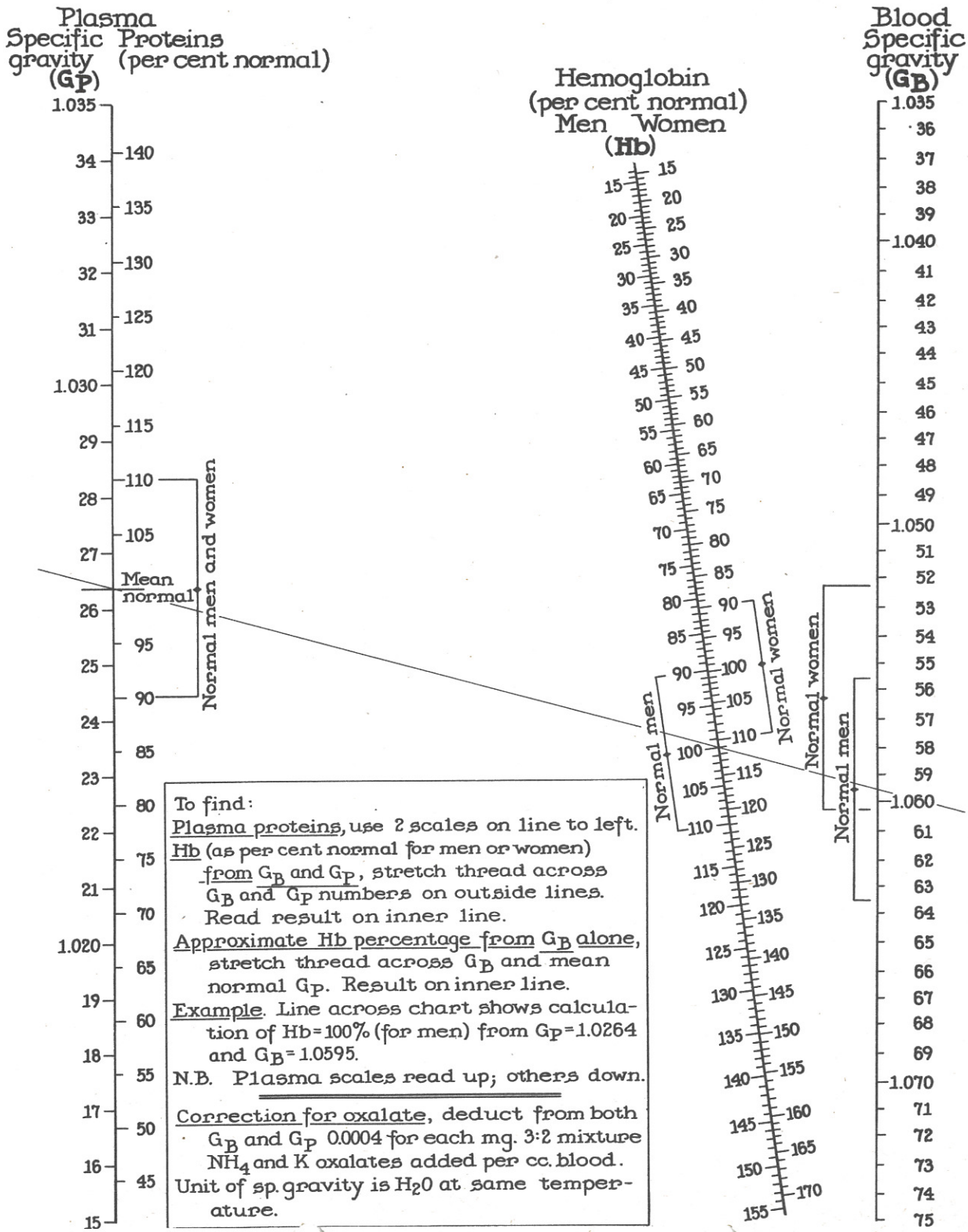


Fig. 2

(Copper Sulfate Method for Sp. Gr. of Blood and Plasma, Phillips, et al.)

Line chart for calculating percentages of normal plasma proteins and hemoglobin from gravities of plasma and blood



Radar, No Evidences of Injurious Effects from: In a Form Letter of August 5, 1942, the Bureau of Medicine and Surgery called attention to the fact that X-rays of the soft type (Grenz rays) are emitted by radar equipment, and requested that radar personnel be examined periodically for evidence of X-ray effects and that a "dental film - paper fastener test" be used to determine roughly the degree of exposure.

During the three months following the distribution of this letter more than 2,000 dental films, exposed as directed, have been received and developed. Only 19 of these showed a definite image of the paper fastener, indicating an exposure of about 0.04 r. This left a wide margin to the 0.1 r. which has been cautioned against by the International X-ray and Radium Committee as harmful.

On November 24, the Bureau of Medicine and Surgery modified its instructions, allowing the development of dental-film tests at the nearest naval activity, with the provision that positive films be reported to the Bureau. So far, the reports have not shown any instances of exposure which definitely could be diagnosed as injurious. A few reports have been received from medical officers calling attention to clinical conditions in radar personnel which possibly could have been due to X-rays. Upon investigation, none of these suspicions have been verified. A study of the personnel at the Radio Materiel School, where radio operators and repairmen are trained, has recently been concluded. The findings of this study were negative for clinical and laboratory evidence of injurious effects.

These data seem to indicate that there is practically no danger of X-ray injuries to radar personnel provided the equipment is shielded and operated as directed. (E.G.H.)

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Pyrethrum-Freon-Insecticide: The importance of the Pyrethrum-Freon-Insecticide bombs in the elimination of insect pests and flying insect vectors of disease is so great that it is desired once again to call attention to the availability of this item. It appears that not only have a number of medical officers failed to understand clearly the availability and proper employment of the Pyrethrum-Freon bombs, but numerous supply officers are unaware that this item has been added as an official item and may be obtained by requisition upon the nearest Naval Supply Depot.

Pyrethrum-Freon-Insecticide is an aerosol mixture. The complete and rapid pervasion of all air space by this mixture is its particular advantage. The insecticide is provided in sizes as follows: 1-pound dispenser, 2 and 5-pound dispensers, and 145-pound cylinders. The 2 and 5-pound dispensers are refillable from the 145-pound cylinders.

The 1-pound dispenser contains sufficient insecticide to kill all mosquitoes in 150,000 cubic feet of space. This insecticide is also effective, although less so, against flies. The concentration of insecticides in the

aerosol is not sufficiently great to be effective against cockroaches and similar crawling beetle-like insect pests. It is believed that all advance bases where flying insects are present as vectors of disease or as pests should be adequately supplied with this insecticide. It is, of course, particularly effective in treating the interior of transport aircraft and should be used when moving from malaria-infested to malaria-free districts or when departing from areas where insect vectors of disease, not present at the flight destination, should be eradicated.

It will be readily seen that the amount required is quite dependent upon existing conditions and not upon the number of men involved. In jungle warfare it has been found an excellent control measure against mosquitoes in the huts of native villagers. It has also been used with good effect in the field, in foxholes and similar excavations.

Pyrethrum-Freon-Insecticide has many advantages: (a) 3 gallons of the ordinary standard spray equals 1 pound of aerosol solution. (b) It occupies only about 1/4 the stowage space. (c) It is noninflammable. In fact it is a fire extinguisher. (d) It requires little or no equipment to operate. (e) All ingredients are nontoxic. (f) It remains suspended 10 to 20 times longer than sprays. (g) One pound will treat 150,000 cubic feet more efficiently than 1 gallon of spray will treat 50,000 cubic feet. (h) There is much greater spread into protected places. (T.J.C.)

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Sulfa Drugs Locally in First Aid: The excellent results obtained by U.S. forces in the field where sulfa drugs have been made available for immediate placement in wounds and are included in every man's pack are well known. The prevention of local infection which has resulted has been without precedent in military surgery. As mass experience has built up, there seems to be no question about the value of such first aid treatment. Practically all wounded men have also had one of the sulfa drugs by mouth - usually sulfadiazine. Metastatic infections and septicemia have been conspicuous by their absence. The U.S. combat experience is paralleled by that of the British.

The Subcommittee on Surgical Infections of the Division of Medical Sciences, National Research Council, has been preparing during the past year a statistical analysis of surgical wound infections which seemed to indicate, in these well controlled studies, that sulfa drugs locally did not prevent local wound infection. It should be emphasized that these observations apply to definitive treatment of wounds rather than to the first aid treatment. The Subcommittee has now adopted as a further project for investigation the local first-aid use of sulfa drugs. The emphasis will be placed upon the immediate introduction of sulfa drugs by ambulance attendants, internes and nurses, wherever the injury occurs. The handling of the patient then would parallel that of combat wounded in which further definitive treatment of the wound is usually delayed 24-48 hours. This investigation will give us a carefully controlled evaluation of the measure, which by mass experience in warfare has proven to be so remarkably effective.

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False Positive Reactions in Serologic Tests for Syphilis: In spite of the fact that the several accepted serologic tests for syphilis, such as the Kahn are remarkably sensitive and specific it is well recognized that false positive serologic reactions do occur. In general these false positive reactions are of three types, technical, general biologic and false positives associated with some diseases.

The technical false positives are due to laboratory errors and occur when the serologic procedures are not given the meticulous care and attention they must have.

A second type of false positive reaction is the reaction of a general biologic (non-luetic) type, which occurs in normal persons who have a reagin-like substance in their serum. Since in the serum of many lower animals there is a substance that gives a positive reaction it was decided to refer to the reaction in human serum, not associated with syphilis or other disease, as the general biologic (non-luetic) type.

A third type of false positive reaction is the type associated with some organic disease other than syphilis, generally an acute infection. As reported in a previous BUMED NEWS LETTER, it has been observed that certain immunizations such as against smallpox and typhoid may occasionally cause transitory false positive serologic reactions. It has been thoroughly established that false positive reactions may occur in malaria, infectious mononucleosis, leprosy, typhus, acute upper respiratory infections, and with other diseases. It has been observed that when the blood specimen is collected when the patient has a febrile condition, the percentage of false positive reactions is greater. In clarifying the incidence of false positive reactions in diseases other than syphilis it is important that the transitory nature of these reactions be recognized. The serologic reactions usually become negative as the patient improves and becomes afebrile.

Since occasional false positive serologic reactions do occur a diagnosis based only upon the results of the serologic tests for syphilis should not be made. Certainly no single positive serologic reaction should be considered as conclusive laboratory evidence of the disease. When a positive serologic report is obtained sufficient recheck tests should be made over a period of several weeks to insure an accurate and dependable serologic study. The establishment of a diagnosis of syphilis on serological grounds alone is not approved by the Bureau (M.M.D. 2287). Reference should also be made to ALNAV of May 10, 1943 reprinted in Bumed News Letter, No. 8.

Positive presumptive Kahn tests should be checked, by standard Kahn test, in the local laboratory. If doubt still exists the serum may then be forwarded to the Naval Medical School for further examination.

When a specimen is sent to the Naval Medical School, Bethesda, Maryland, for recheck and verification procedures, it is requested that the specimen be two 5 cc. sterile, unheated, serum specimens accompanied by laboratory report form, and be sent special-delivery air mail. Such specimens will be sent to Dr. Kahn's laboratory for further verification studies when necessary. (R.D.T.)

Convalescent Hospitals for the Treatment of Psychiatric Combat Casualties: The attention of all medical officers is invited to a proposed innovation in psychiatric rehabilitation. Especially is it desired that the general medical officer as well as the psychiatrist be informed of this move and the purpose underlying it so that they may be better prepared to recommend patients suitable for admission to these institutions.

The Surgeon General has authorized the establishment of convalescent hospitals for the treatment of psychiatric casualties resulting from combat situations. These hospitals are now in the process of organization. Patients diagnosed as combat or operational fatigue, psychoneurosis or war neurosis are those considered amenable to treatment in these institutions. The purpose of the program may be largely defeated unless the patients transferred to the hospitals are properly selected. It is felt, therefore, that a few words on the type of patient for which these hospitals are designed may be of value.

The treatment program is planned to cover a maximum of 4-6 weeks. It is hoped that the major proportion of such patients can be returned to active duty. The great stress to which troops in this war are subjected induces combat reactions which have both psychological and physiological consequences. Because of the differences inherent in the nature of individual patients, there are also differing reactions to such stress situations. Those whose reactions spring from a pre-combat psychiatric background are not suitable for admission to this type of convalescent house.

The patient deemed most satisfactory for this treatment fulfills the criteria for the diagnosis of combat or operational fatigue in that he shows: (1) A stable personality prior to the appearance of symptoms; i.e., no evidence of gross maladjustment in childhood or adolescence and a previous service record of competence and stability; (2) a history of stresses or experiences in combat or operational areas of sufficient intensity to act as the likely precipitating factor; (3) objective evidence of anxiety; repeated anxiety nightmares; associated autonomic nervous system dysfunctions (tachycardia, increased sweating, gastro-intestinal disturbance, etc.); heightened startle response to stimuli, such as noise or movements; (4) probable recoverability following rest, physiological care and short-term re-educative psychotherapy.

The type of treatment which will be conducted is designed along the lines of group management. In addition to physiotherapy and recreation there will be planned group psychotherapy. Group psychotherapy presents a number of advantages over other forms of psychotherapy and is valued for these advantages as well as for its efficiency in handling numbers of men. A single psychiatrist can simultaneously see as many as twenty-five patients in the time ordinarily consumed in one interview. Individual therapeutic sessions may be added if they are particularly indicated. This form of collective treatment can be completely fitted to a daily twenty-four-hour regimen in such an atmosphere where the situation can be purposefully controlled. Group therapy is an educational procedure which promotes, by gradual participation, the acquisition of insight.

Group discussions, in the nature of round-table conferences, may be conducted several times daily. The prevailing attitude is to be one of frank inquiry and complete freedom of expression; the purpose being to give each man the opportunity to participate in the discussion of the principles of mental hygiene and in that way to gain personal benefit. A planned series of consecutive discussions will include indoctrinational lectures on the purpose and function of the Navy, also what is personally most important to the patient; the discussions will take up the problem of reactions which are common to all combatants before and after battle. As a consequence of this cooperative endeavor, the group will become an animated, integrated unit operating in a "let's get well" atmosphere.

The general feeling of being unique in the possession of particular symptoms is dissipated when the patient learns that others also have similar complaints. In this way the stigma, which unfortunately all too frequently is attached to ordinary psychoneurotic reactions may be avoided. Intensive studies of combat fatigue have clearly shown that a too-penetrating analysis of individual reactions often serves to fix symptoms rather than to dissipate them. The aim throughout will be to restore the patient to a pre-combat level of efficiency.

Since each patient has come from a military unit and since each has received his disability in the prosecution of a common military objective, and particularly since the nature of his incapacitating disability is similar to those of others, it is appropriate that he should be grouped with others and treated collectively.

Two favorable results are expected. The first is a high recovery rate, the second and quite as important is that those who may be found unfit to return to active duty may be salvaged from the psychoneurotic group and returned to society, not as burdens, but as helpful and productive citizens. (W.F.K.)

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Recovery of Wounded: Announcement that more than 97 per cent of Naval and Marine wounded from Pearl Harbor to March 31, 1943, have recovered was made on May 19 by the Officer of War Information. These figures were contained in a report by OWI on the care of the wounded by the medical departments of the Army and Navy.

Percentage figures for the recovery of Army wounded are not available at present owing to incompleteness of records from the fighting fronts. An analysis of available data on Army wounded shows that recoveries are comparable to Naval and Marine percentages.

Of all Navy and Marine personnel wounded only 2.6 per cent died subsequently. Fifty-three per cent were returned to duty. Still under treatment as of March 31 were 43.5 per cent. Invalided from service were 0.9 per cent.

The breakdown of the figures shows: Naval officers wounded, 61.6 per cent returned to duty; 35.9 per cent were still under treatment; .2 per cent were invalided from service; only 2.3 per cent died.

Of Naval enlisted men wounded, 60.4 per cent returned to duty; 35.4 per cent were still under treatment; 1.4 per cent were invalided from the service; and 2.8 per cent died.

Of Marine officers wounded, 46.8 per cent returned to duty; 51.6 per cent were still under treatment; and 1.6 per cent died. None was invalided.

Of Marine enlisted men wounded, 41.5 per cent returned to duty; 55.9 per cent were still under treatment; .4 per cent were invalided from service; and 2.2 per cent died.

In the original occupation of North Africa, the only deaths were those of men killed outright or so badly wounded that nothing could have saved them. This was also true in other theaters of war.

Never before in the history of the world has the fighting man had available the medical care and equipment the United States now furnishes its defenders. When medical supplies are delayed in reaching the front--and not even a Red Cross can stop a bomb, as the Nazis and Japs know well--our doctors are trained to perform their duties with whatever equipment is at hand. In the North African campaign, ships carrying medical material were torpedoed. Yet our medical care system was established right from the beaches of the Mediterranean, and the hundreds of recoveries from wounds testify to its effectiveness. (Army and Navy Register, May 22, '43.)

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The Naval Hospital Fund: Legislation has been proposed to abolish the time honored Naval Hospital Fund. The bill establishing the Naval Hospital Fund was signed by President John Adams on July 16, 1798, the provisions of which authorized the familiar deduction of $0.20 per month from the pay of officers and men of the Navy and Merchant Marine Service. Hearings before the Senate and House Committees on Naval Affairs have been held and both committees have acted favorably. (J.A.M.A., June 5, '43.)

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ALNAV #99 - 15 May '43: In order to comply with article twenty-one of the Geneva Conference, all medical personnel, male and female, both officer and enlisted including medical, dental, hospital and nurses corps, will have stamped on the identification card N Nav 546 in the lower left corner, a red cross of appropriate size. In addition, the date of issue, rank or rating and corps will be added directly beneath the photograph. Medical personnel are directed to carry the identification card on their person at all times.

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Index, Volume 1, Bumed News Letter, Numbers 1-9, for period March 5 to June 25 inclusive. Abbreviations indicate type of matter indexed as follows: "E," editorial; "ab," abstracts, medical journals; "R," abstracts, reports of current research; the star (*), an original item. Numerals indicate number and page in Volume 1.

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May 23, 1943

JOINT LETTER

From: The Chief of the Bureau of Medicine and Surgery.
The Chief of Naval Personnel.
To: All Ships and Stations.

Subject: Filariasis (Wuchereria bancrofti), administrative control of infected personnel.

Enclosure: (A) Geographic Distribution of Wuchereria Bancrofti.

1. It is generally recognized that the symptoms of filariasis caused by W. bancrofti, whether in early or late stages, have a tendency to recur in spite of any treatment now available. It is also known that the symptoms are likely to increase in severity with continued reinfections, and that disabling complications develop in persons who reside in endemic areas and are exposed to repeated infections. It has been observed, however, that when an infected person is removed from an endemic area to a temperate or cold climate, the symptoms ameliorate and ultimately disappear without disabling complications.

2. The disposition of personnel returned to the United States on account of filariasis has been discussed with the U.S. Public Health Service, and the opinion formulated that the risk of introducing this disease among the civilian population of the United States is not sufficient to warrant restrictions on the movement of infected individuals except that those with symptoms should be hospitalized until clinically free of the disease.

3. In view of the above considerations, the following instructions should guide the administrative control of the Navy and Marine Corps personnel with filariasis due to W. bancrofti:

(a) Personnel who acquire filariasis shall be transferred from endemic areas to the nearest U.S. Naval Hospital in the United States. The transfer is to be accomplished at the earliest practicable date.

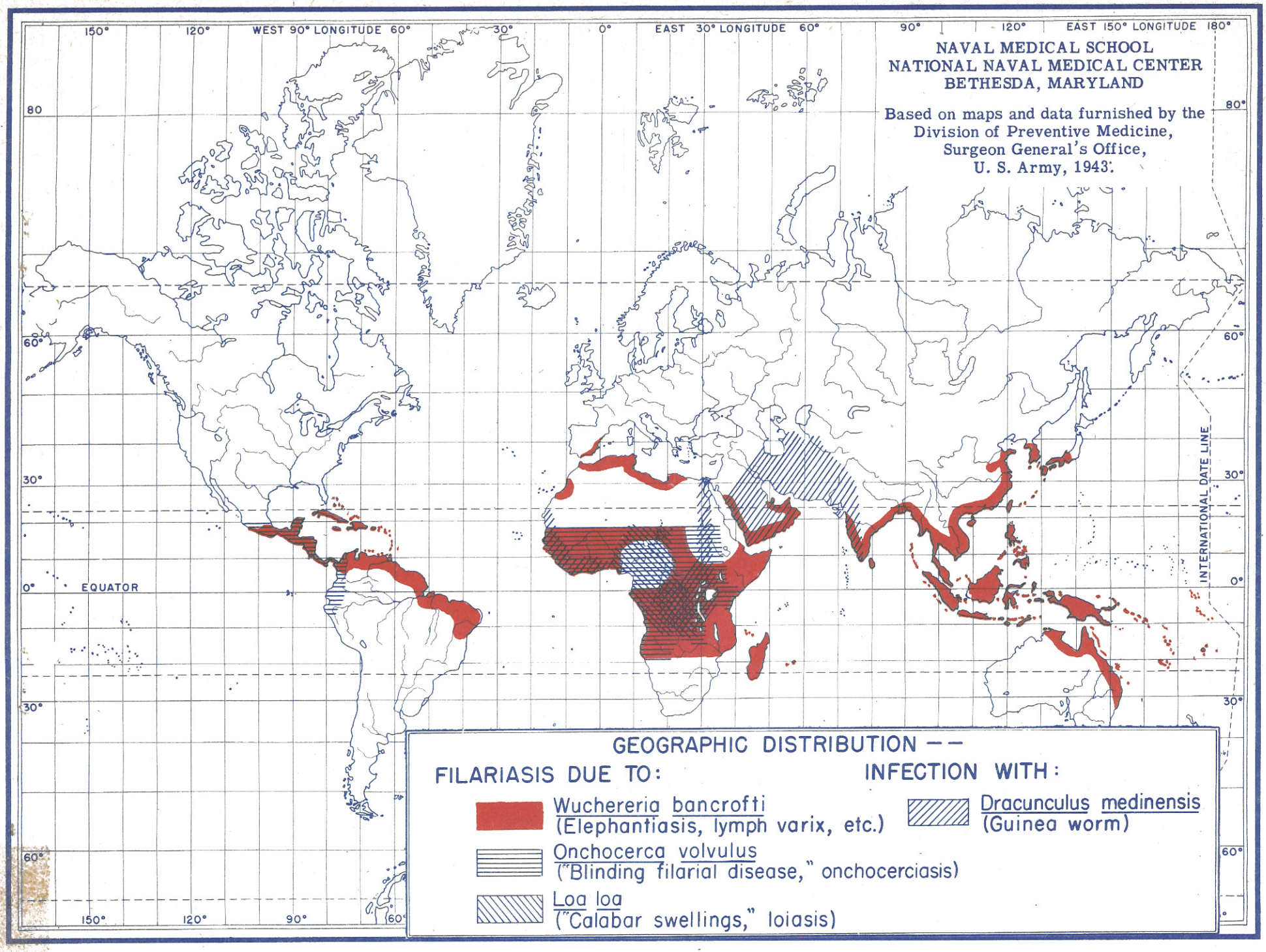
(b) Personnel with symptoms of filariasis shall be hospitalized until clinically free of the disease. Such hospitalization shall be within the continental United States whenever possible.

(c) Infected personnel shall not be sent again into endemic areas. An entry to that effect shall be made in the health record, and in case of enlisted personnel also in the service record.

(d) The presence of microfilaria in the blood, in the absence of clinical symptoms, shall not warrant restriction on the movements of the infected individuals except as noted in (c) above.

4. The geographical distribution of filariasis due to W. bancrofti is roughly shown. Enclosure (A).

RANDALL JACOBS ROSS T. McINTIRE


NAVAL MEDICAL SCHOOL
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND
Based on maps and data furnished by the Division of Preventive Medicine, Surgeon General's Office, U. S. Army, 1943.
WEST 90° LONGITUDE 60°
EAST 30° LONGITUDE 60°
EAST 150° LONGITUDE 180°
EQUATOR
INTERNATIONAL DATE LINE
GEOGRAPHIC DISTRIBUTION --
FILARIASIS DUE TO:
Wuchereria bancrofti
(Elephantiasis, lymph varix, etc.)
Onchocerca volvulus
("Blinding filarial disease," onchocerciasis)
Loa loa
("Calabar swellings," loiasis)
INFECTION WITH:
Dracunculus medinensis
(Guinea worm)